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Supplemental Information

Assembly of eIF3 Mediated by Mutually Dependent Subunit Insertion

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Supplemental Information

Supplemental Experimental Procedures

Isolation of eIF3 complexes and sub-complexes from N. crassa lysates

Strains containing tagged eIF3 subunits were grown in liquid culture (Vogel's minimal media, 2% sucrose) for 40-46 hours from an initial conidial density of OD_{600} = 0.04. Mycelia were collected using a Büchner funnel with filter paper, rinsed briefly with deionized water and immediately transferred to dry ice. Frozen biomass was ground up using a mortar and pestle in liquid nitrogen. Lysis buffer (TBS, 0.5% Triton X-100, 10% glycerol, 2 mM EDTA and protease inhibitors (Roche 05 056 489 001)) was added directly to the cold mortar containing the powered biomass (~3-5 mL lysis buffer per gram of biomass) and further ground with the mortar and pestle until the lysate was an even consistency. Typically 10-30g of damp biomass was used for each assay. The lysate was further mixed at 4 °C using a magnetic stir bar for 10 min. The lysate was cleared by centrifugation and filtered with a 0.2 µm syringe filter. Anti-FLAG affinity beads (Sigma A2220) were added (~200 µl bed volume) directly to the lysate for 4-20 hours at 4 °C, collected by centrifugation and washed 5 times each with ~10 bed volumes of wash buffer (TBS, 10% glycerol). The complex was eluted using FLAG peptide (Sigma 4799). The eluate was washed three times with ~10 volumes of wash buffer containing 1 mM DTT though a Millipore centrifugal filter unit (10,000 MWCO) to remove FLAG peptide. Eluates were analyzed by 12% SDS-PAGE, stained with Coomassie blue or SYPRO (Thermofisher S-12000) ruby stain using the manufacturer's protocol. The composition of eIF3 samples purified from *N. crassa* were further analyzed by tandem mass spectrometry (LC MS/MS) as previously described

(Smith et al., 2013). Samples analyzed by electron microscopy were further purified using Biospin 6 columns (Biorad) into TBS with 5% glycerol.

Human eIF3 immunoprecipitations and westerns blotting

FLAG-tagged and un-tagged eIF3 subunits were all cloned into the plasmid nLv-103 (Addgene) using In-Fusion cloning (Clonetech). Constructs with N-terminal FLAG-tags contained identical FLAG-tag sequences as the *Neurospora* constructs created in this study, which were added by PCR.

HEK 293T cell lines (wild-type or eIF3h KO) were grown at 37 °C, 5% CO_2, in 6-well plates with Dulbecco's Modified Eagle Medium (DMEM) to a confluency of ~60% for transient transfections. Cells were transfected using Lipofectamine 2000 (ThermoFisher) by following the manufacturer's protocol and 1-2 ug of total plasmid DNA per culture, then grown for 20-30 hours after transfection.

Cells for western blots were rinsed with 1 mL of Hank's balanced salt solution (Gibco) and detached using 0.5 ml 0.05% trypsin (Gibco). Cell pellets were stored at -20 °C until lysis. Cell pellets were lysed using 250 µL of lysis buffer (TBS, 0.5% Triton X-100, 10% glycerol, 2mM EDTA) on ice. Lysates were cleared by centrifugation at 4 °C. A 50 µL aliquot of the cleared lysate was removed for lysate (L) fractions analyzed by western blot. The remainder of the lysate was incubated with ~20 µL bed volume of anti-FLAG affinity beads (Sigma), rotating for two hours at 4 °C. Unbound sample in the lysate (FT) was removed and saved for western blots. Beads containing FLAG-tagged eIF3 subunits and other bound proteins were washed three times with 200 µL of wash buffer (TBS + 10% glycerol). Wash fractions (W) were pooled and concentrated down to 200 µL prior to western blotting. Proteins were eluted (IP) using serial

elutions of 3 x 50 µL elution buffer (Wash buffer + 1 µg/µL FLAG peptide). Samples were separated using 12% SDS PAGE for western blotting. Samples for the FLAG-tagged eIF3e-ΔC immunoprecipitation were further analyzed by LC MS/MS as described (Smith et al., 2013).

Complexes of eIF3 were immunoprecipitated from wild-type or eIF3h KO HEK 293T cells as described above, except Dynabeads (Thermofisher) with protein-G coupled eIF3b primary antibody (Bethyl) were used to pull down endogenous eIF3 complexes from cellular lysates. Proteins were transferred to PVDF membrane using standard protocols. Membranes were blocked for one hour at room temperature using 5% non-fat milk in TBS-t (TBS + 0.05% Tween-20). Primary rabbit antibodies for eIF3 subunits (Bethyl) were incubated for 1-2 hours at room temperature in TBS-t with 1% milk. Secondary antibody treatments were also carried out at room temperature with TBS-t + 1% milk for 1-2 hours. Membranes were washed three times with TBS-t before adding HRP substrate and imaging.

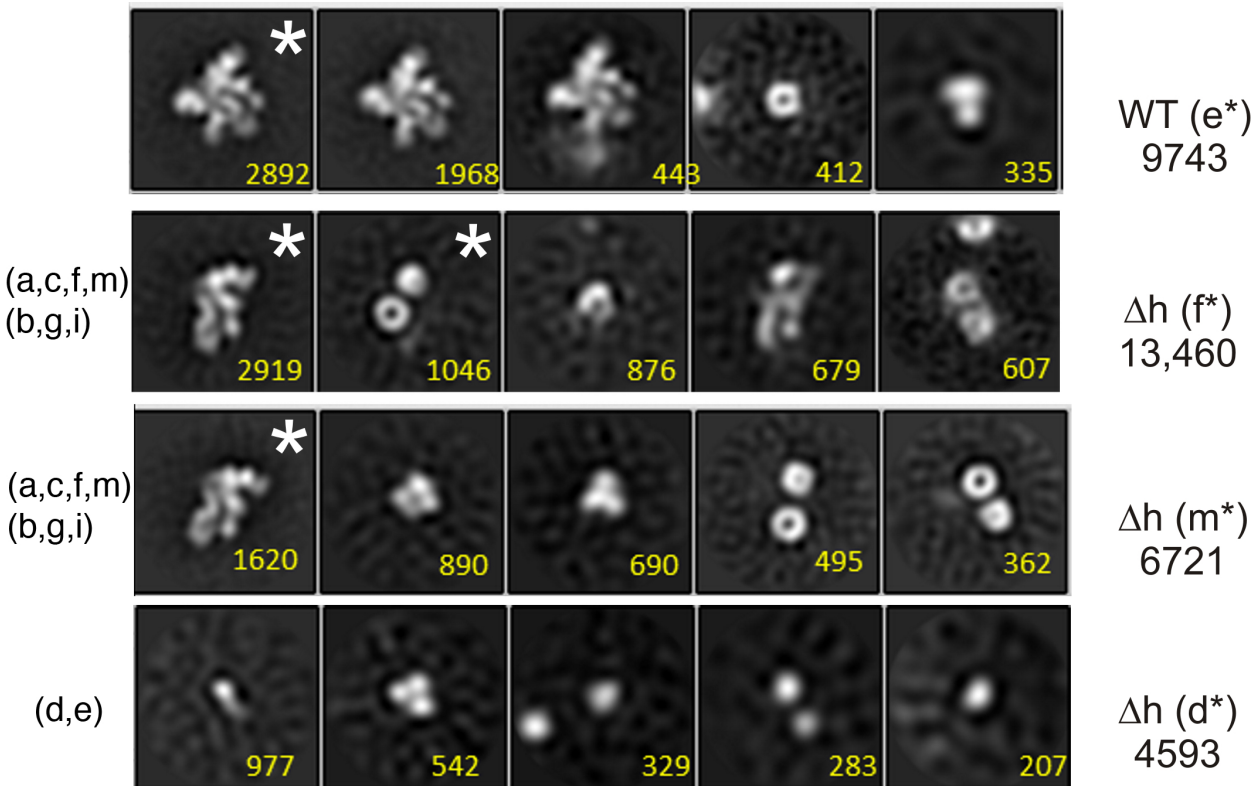

Figure S1. Related to Figures 1 and 3 – 2D class averages for negative stain EM images of eIF3 and eIF3Δh.

Negative stain images of the top five class averages for wild-type eIF3 (WT) and eIF3Δh (Δh) sub-complexes. Subunits bearing the FLAG affinity tag are indicated in parentheses with an asterisk. Subunits included in the eIF3Δh sub-complexes are determined by SDS PAGE and indicated to the left (Figure 1). Total particles picked for each class are indicated below each complex name to the right. White asterisks indicate classes used in 2D difference maps as well as for modeling the crystal structures of WD-domains of eIF3b and i in Figure 3C-3E.

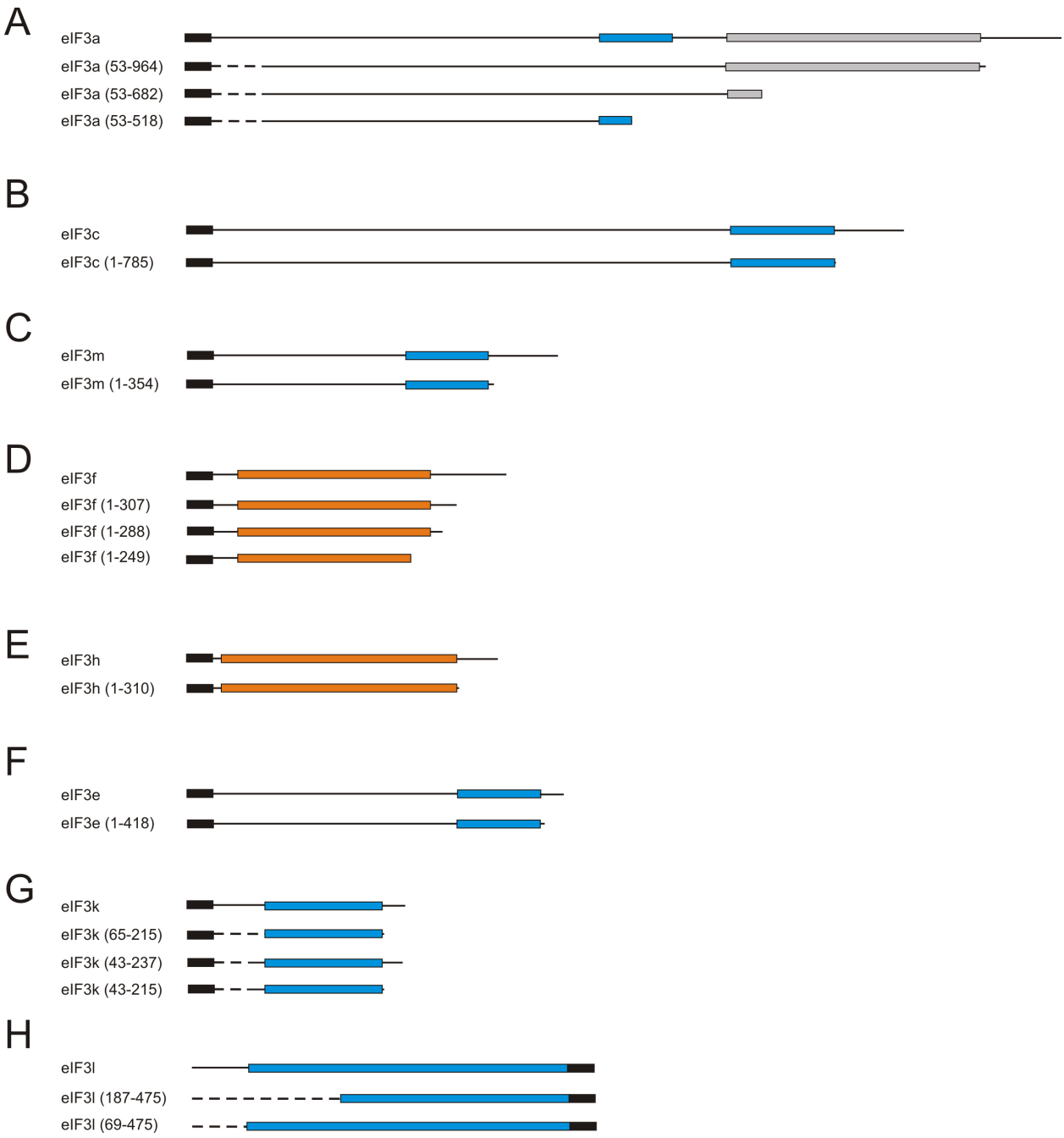

Figure S2. Related to Figures 1 and 5 – Schemes of truncated *N. crassa* eIF3 subunits used in this study.

Schemes of full-length and truncated eIF3 constructs. The FLAG tag, PCI, MPN and spectrin coiled-coil domains are represented with black, blue, orange and grey rectangles, respectively. Subunits and amino acid ranges of the truncations are indicated. Panels A-H indicate respective

SDS PAGE gels of eIF3 sub-complexes (Figure S3 A-H) and modeled binding surfaces (Figure S4 A-H) for each subunit. Note that the C-terminal truncations of eIF3a were based off of an older annotation of *Neurospora* eIF3a (NCBI reference sequence XP_956151.2) missing the 52 N-terminal amino acids in the updated annotation (NCBI reference sequence XP_956151.3). These 52 amino acids have no effect on eIF3 assembly (Figure S3A).

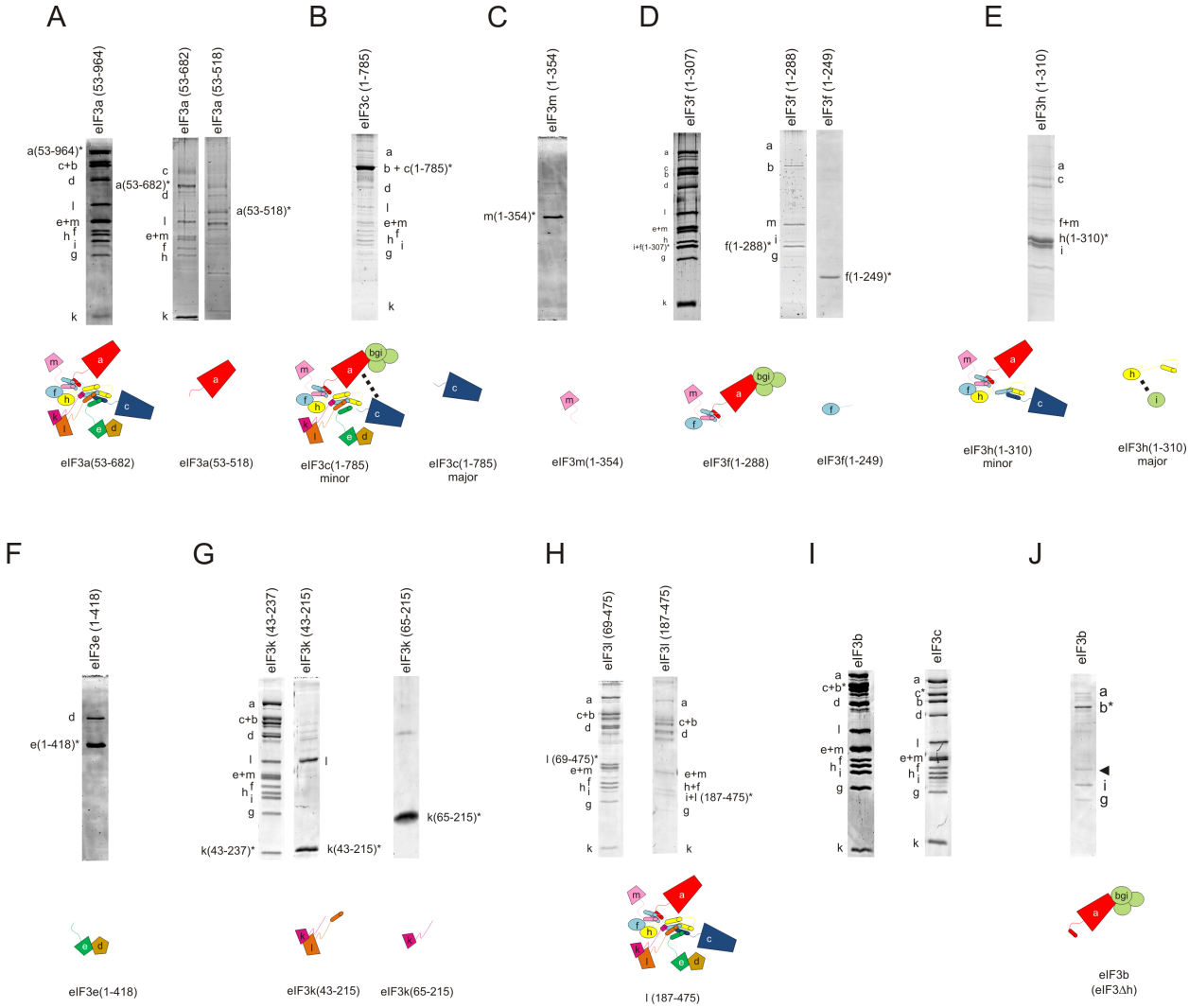

Figure S3. Related to Figures 1 and 5 – Purification of eIF3 sub-complexes from *N. crassa* using truncated subunits.

(A-H) SDS PAGE gels of eIF3 sub-complexes pulled down by the truncated eIF3 subunit containing a FLAG-tag, indicated over each gel. Each panel (A-H) corresponds to its respective truncation scheme (Figure S2 A-H) and modeled binding surfaces (Figure S4 A-H). Schematics of each purified subunit or sub-complex are shown below each panel. The tagged subunit truncation is indicated below each scheme. (I) Complexes of eIF3 purified using subunits with N-terminal FLAG-tags, indicated above each gel, from wild-type *N. crassa*. (J) Purification of a sub-

complex composed of eIF3a, b, g and i with a scheme of the sub-complex below where eIF3b is tagged in an eIF3Δh background. The arrow signifies an unidentified protein that does not co-migrate with any eIF3 subunit. Subunits identified by size and LC MS/MS are indicated next to gels. The tagged subunit is marked with an asterisk.

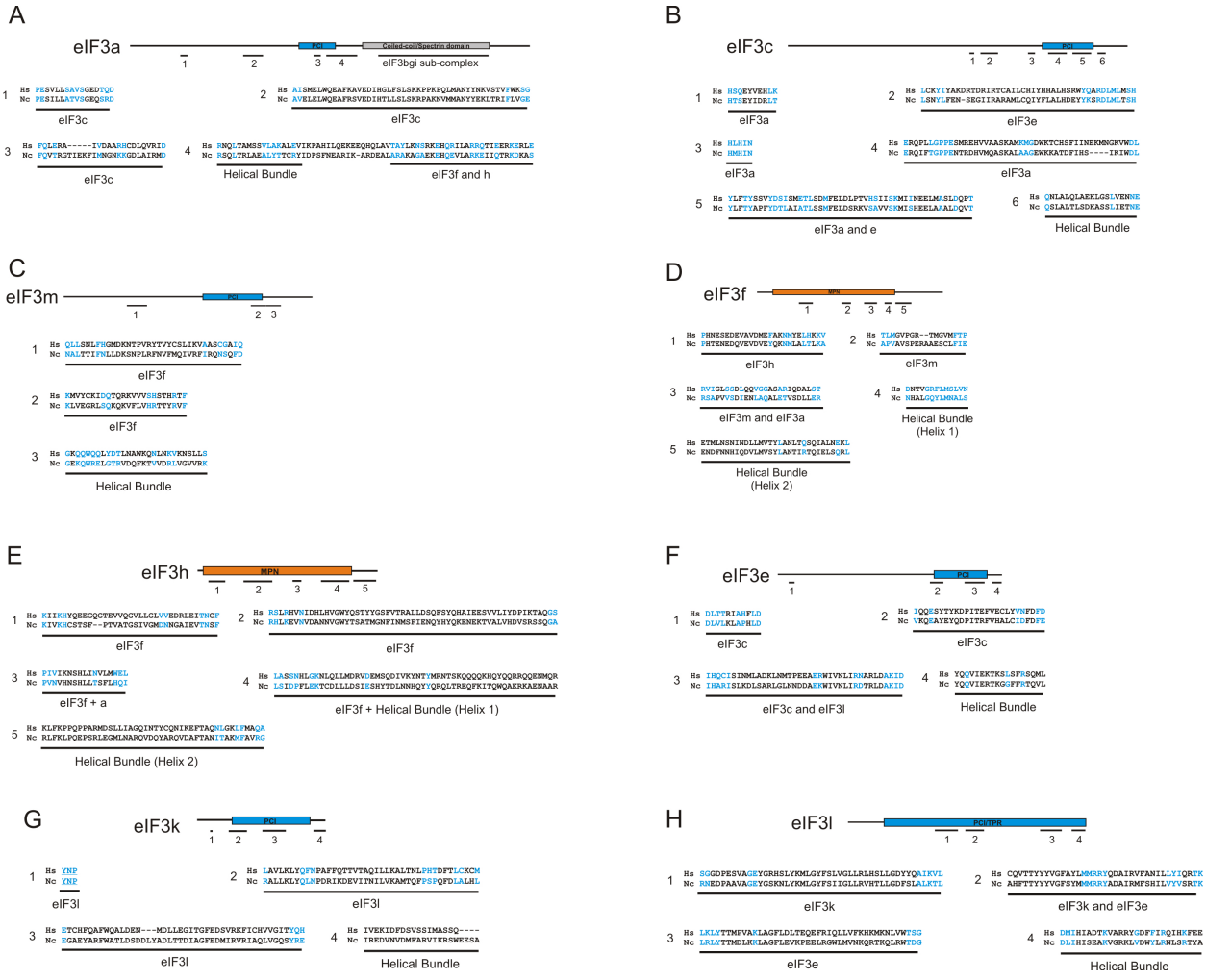

Figure S4. Related to Figures 1 and 5 – Modeled binding surfaces for eIF3 subunits.

Modeled binding surfaces for eIF3 subunits based on the cryo-EM reconstruction of human eIF3 (PDB 5A5T) (des Georges et al., 2015). Each subunit is indicated next to the scheme. Panels (A-H) correspond to their respective truncation scheme in Figure S2 and purified sub-complexes in Figure S3. Lines with numbers indicate predicted binding surfaces across the subunit. The sequences of the interaction surfaces are numbered in the order of the alignments between human (Hs) and *N.crassa* (Nc) sequences shown below. Blue amino acids are potentially involved in binding and are highly conserved in multi-cellular organisms including *Neurospora*

crassa (des Georges et al., 2015). Binding partners for each surface are indicated below the alignment.

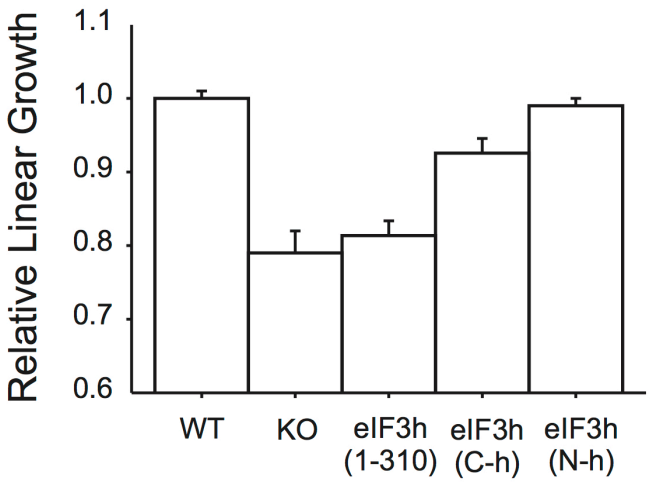

Figure S5. Related to Figure 1 – The MPN domain of eIF3h is not sufficient to restore the growth phenotype of the eIF3Δh strain.

Bars represent relative linear growth of *N. crassa* strains: Wild-type (WT), eIF3Δh strain (KO), eIF3Δh strain expressing only the MPN domain of eIF3h (eIF3h 1-310), eIF3Δh strain expressing full length eIF3h with either N-terminal (eIF3h N-h) or C-terminal (eIF3h C-h) FLAG-tags. Relative linear growth is the fraction of wild-type growth (see experimental procedures). Error bars represent the standard deviation of three or more experiments.

Table S1. Related to Figure 4. Identification of proteins co-immunoprecipitating with eIF3e ΔC-term by LC MS/MS

Accession	Protein	Peptides
P60228	Eukaryotic translation initiation factor 3 subunit E	14
O15371	Eukaryotic translation initiation factor 3 subunit D	12
P07437	Tubulin beta chain	5
O14744	Protein arginine N-methyltransferase 5	4
Q9BQA1	Methylosome protein 50	4
P60709	Actin, cytoplasmic 1	3
P68363	Tubulin alpha-1B chain	3
Q07021	Complement component 1 Q subcomponent-binding protein, mitochondria	2
P68104	Elongation factor 1-alpha 1	2
P48741	Putative heat shock 70 kDa protein 7	2